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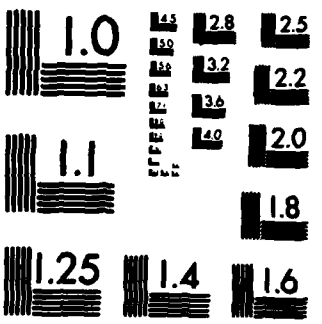
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Stern College for Women
Yeshiva University
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Transport of Ca^{2+} Across Phosphatidylcholine Vesicles

Lea Blau

1983

Last year's report included results on the Ca^{2+} transport induced by the naturally occurring polyether carboxylic acid ionophore A23187. A second order dependence of the initial rate of Ca^{2+} efflux from egg phosphatidylcholine (PC) large unilamellar vesicles (LUV) was established. An attempt was made to establish the dependence of the rate on Ca^{2+} concentrtion by varying the amount of Ca^{2+} trapped in the vesicles. This method did not give good results.

Since the last progress report the rate dependence on Ca^{2+} concentration was found to be first order. This study has been conducted in LUV containing the Ca^{2+} sensitive dye arsenaro III (Weissman, et al, 1980) and adding varying amounts of Ca^{2+} to the external medium. The initial rate of Ca^{2+} influx was calculated from the tangent, at zero time, to the curve obtained by following the absorbance changes at 650 nm as a function of time. The data obtained at various Ca^{2+} concentration are summarized ~~in Table I~~.

TABLE I

The Effect of Ca^{2+} Concentration on the Initial Rate of Ca^{2+} Influx into LUV.

Ca^{2+} (10^{3} M)	$10^{2} \Delta A_{660}$ /min
3.1	2.1
4.3	2.8
6.2	4.4
9.2	6.3
12.4	9.0
15.4	11.0
18.5	13.2

The vesicles were 0.12mM egg PC and the internal arsenazo III was 7.6mM. The reaction was carried out at 22°.

It is evident from the above given data that there is a linear increase of the initial rates of Ca^{2+} influx with increase in Ca^{2+} concentration. Our data are in agreement with previous findings, using different vesciles (Pohl, et al. 1980), that the Ca^{2+} is transported across the PC bilayers as an electroneutral complex $(A23187^{-})_2 Ca^{2+}$.

In order to investigate the role of the ionizable carboxylic acid of A23187 in Ca^{2+} transport, its methyl ester (CH_3 A23187) was prepared. This compound was found to be much less effective in transporting Ca^{2+} (Table II)

TABLE II

The Effect of CH_3 A23187 Concentration on the Initital Rate of Ca^{2+} Efflux from LUV

CH_3 A23187 (10^6 M)	$10^3 \Delta A_{650}$/min
2.1	4.2
2.6	6.0
3.1	6.8
3.6	7.0
4.1	10.0
5.2	12.0
6.2	12.0
7.7	17.0
15.5	39.0

The PC concentration was 0.060mM and the internal Ca was 0.10M. The temperature was 22 degrees .

A log-log plot of the data from Table II yielded a straight line with a slope of 1.08 $\pm$ 0.06 indicating a first order dependence of Ca^{2+} efflux with respect to CH_3 A23187 concentration. It is assumed therefore that the Ca^{2+} is transported as an electrogenic complex.

Another polyether ionophore Lasalocid or X537A was investigated. This compound transports a variety of mono- and divalent cations. It was found that it has a lower ionophorous activity than A23187. The results are shown in Table III.

TABLE III

The Effect of X537A Concentration on the Initial Rate of Ca^{2+} Efflux from LUV

X537A (10^6 M)	10^3 ΔA_{650}/min
0.52	2.3
0.9	3.4
1.2	4.5
1.7	6.9
2.9	11.5
3.5	15.0
5.8	26.0

The PC concentration was0.076mM and the internal Ca^{2+} concentration was 0.10M. The tempeature was 22°

A log-log plot of initial rates vs. the concentration of the ionophore yielded a straight line with a slope of 0.95 ± 0.05. The experiment was repeated several times with the sodium salt of X537A and the free acid. In each case the slope was close to one with an error limit of about 10%. Therefore, in contradiction with previous findings (Kafka and Holz, 1976) our results indicate a first-order dependence on X537A. Similar results were suggested at concentrations lower than 5μM ionophore by others (Hyono et al.

1975). By using arsenazo III-trapped vesicles a first order dependence of the Ca^{2+} influx on Ca^{2+} concentration was established (when the Ca^{2+} concentration was increased from 10 to 20mM the influx rate increased from 0.0005 to 0.0010 absorbance units at 650nm/min.). These results support the existence of an 1:1 X537A:Ca^{2+} complex which moves across the membrane.

The initial rates of Ca^{2+} transport mediated by CH_3A23187 and X537A were extrapolated to 0.096 μM and the values obtained were compared in Table IV with rates induced by A23187.

TABLE IV

Comparison of Ca^{2+} Transport Rates Induced by Various Ionophores

Ionophore (0.096 μM)	Initial Rate $10^3 \Delta A_{650}$/min	Relative Rate
A23187	49	100
X537A	0.39	0.8
CH_3A23187	0.22	0.5

The above comparison indicates that the ionophores differ, besides in their stoichiometry of the complex being transported across the membrane, also in their ionophorous potency.

II. Transport of K^+ Across Phosphatidyylcholine Vesicles

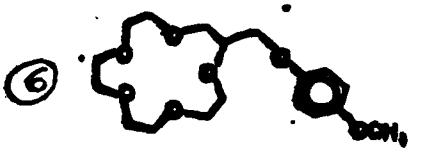
A. Ion Sensitive Electrode Measurements

The K^+ efflux from LUV, mediated by valinomycin was followed by using an ion sensitive electrode. Initial rates were estimated by plotting the increase in $[K^+]$ as a function of time. The study was carried out with Orion Ionalyzer Model 901 equipped with a printer. Readings were taken every 6 seconds at the start of the reaction and later at 0.5 min intervals. In a similar fashion initial rates of K^+ efflux induced by a series of Lariat ethers (structures given in Table V) were measured (Talbe IV). The rates

TABLE V

The structures of lariat either supplied by the laboratory of Dr. George W. Gokel

Carbon-pivot lariat ethers



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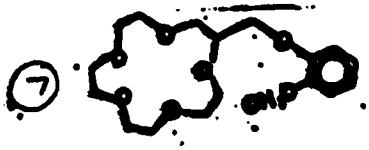
2-((4-methoxyphenoxy)methyl)-15-crown-5



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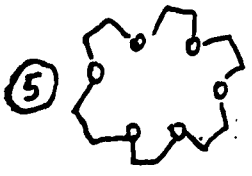
2-((2-methoxyphenoxy)methyl)-15-crown-5



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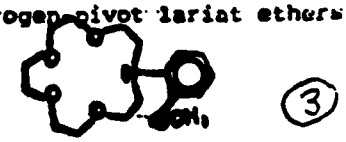
18-Crown-6

Nitrogen-pivot lariat ethers



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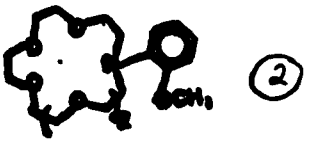
N-(2-methoxyphenyl)monoaza-15-crown-5



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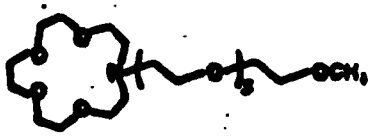
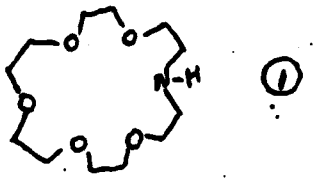
N-(2-methoxyphenyl)monoaza-18-crown-6



N-(O-methyltetraethyleneoxy) monoaza-15-crown-5



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Monoaza-18-Crown-6

were followed at 0.33mM lariat ether and 3 μM valinomycin. The results obtained with valinomycin were multiplied by an adjustment factor 330/3 for comparison purposes.

TABLE VI

Compariosn of initital rates of K^+ efflux from LUV mediated by various ionophores

Ionophore	Blank	Valinomycin	1	2	3	4	5	6	7
Relative rates of K^+ efflux	0.1	100	0.2	2.5	0.6	0.1	0.1	0.4	1.3

PC Concentration - 0.75 $\pm$ 0.05mM: Intravesicular

K^+ Concentration - 0.15M

B. Lipophilic Anion Movement

The fluorescent dye carboxyfluorescein (CF) is a liophilic anion at pH 7.0. Under normal conditions it does not permeate the phosphiolipid bilayer rapidly. However in case of transport of a positively charged complex CF permeability may be accelerated (Shanzer et al, 1983).

Preliminary results show that small unilamellar vesicles containing CF and K^+ became leaky to CF in presence of valinomycin. Dialysis of the

vesicles into an external medium containing isotonic choline chloride solution yielded an efflux of 26% CF in presence of ethanol and 54% when ethanolic valinomycin was added (final concentration of valinomycin in dialyzing sample 0.50 μM) after 18 minutes of dialysis, and 31% of the trapped CF leaked out in the presence of ethanol and 99% in presence of vainomycin after 35 minutes. The extent of leakage of CF in presence of 0.3% ethanol seems high and the experiments should be repeated.

The measurements of ionophore-mediated K^+ transport are preliminary and further work is requied. The research will be directed toward improvement of methods of measurements and seeking more effective ionophores in the lariat ether series.

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Abbreviations: PC - phosphatidylcholine; LUV - large unilamellar vesicles; CF - carboxyfluorescein. CH_3A23187 - methylester of A23187

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